Kunutsor, S. K., Flores-Guerrero, J. L., Kieneker, L. M., Nilsen, T., Hidden, C., Sundrehagen, E., Seidu, S., Dullaart, R. P. F., & Bakker, S. J. L. (2018). Plasma neutrophil gelatinase-associated lipocalin and risk of cardiovascular disease: Findings from the PREVEND prospective cohort study. *Clinica Chimica Acta*, *486*, 66-75. https://doi.org/10.1016/j.cca.2018.07.034

Plasma neutrophil gelatinase-associated lipocalin and risk of cardiovascular disease: findings from the PREVEND prospective cohort study

Setor K. Kunutsor PhD[a,b,*], José L. Flores-Guerrero MD[c], Lyanne M. Kieneker MSc[c], Tom Nilsen MSc[d], Clara Hidden PhD[d], Erling Sundrehagen PhD[d], Samuel Seidu MD[e,f], Robin P.F. Dullaart PhD[c], Stephan J.L. Bakker PhD[c]

[a]Translational Health Sciences, Bristol Medical School, University of Bristol, Southmead Hospital, Learning & Research Building (Level 1), Bristol, UK

[b]National Institute for Health Research Bristol Biomedical Research Centre, University of Bristol, Bristol, UK

[c]Department of Internal Medicine, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands

[d]Gentian, Moss, Norway

[e]Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, Leicester, LE5 4WP, UK

[f]Diabetes Research Centre, University of Leicester, Leicester General Hospital, Gwendolen Road, Leicester, LE5 4WP, UK.

***Corresponding Author,** Translational Health Sciences, Bristol Medical School, University of Bristol, Southmead Hospital, Learning & Research Building (Level 1), Bristol, UK; Fax: +44-1174147924; Phone: +447539589186; Email address: skk31@cantab.net

Abbreviation: AKI, acute kidney injury; AMI, acute myocardial infarction; BMI, body mass index; CABG, coronary artery bypass grafting; CHD, coronary heart disease; CHS, Copenhagen Heart Study; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CI, confidence interval (CI); CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FAR, floating absolute risk; FPG,

fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; IHD, ischemic heart disease; IQR, interquartile range; MMP, matrix metalloproteinase; NGAL, Neutrophil gelatinase-associated lipocalin; PREVEND, Prevention of Renal and Vascular End-stage Disease; PTCA, percutaneous transluminal coronary angioplasty; RBS, Rancho Bernardo Study; SD, standard deviation; SBP, systolic blood pressure; UAE, urine albumin excretion

ABSTRACT

Background: Neutrophil gelatinase-associated lipocalin (NGAL), a novel biomarker of acute kidney injury, might play a role in the development of atherosclerotic cardiovascular disease (CVD). We aimed to assess the association of circulating NGAL with CVD risk.

Materials and methods: Plasma NGAL concentrations were measured at baseline in 5,275 participants in the PREVEND prospective study. Hazard ratios (95% confidence intervals [CI]) for CVD were estimated.

Results: After a median follow-up of 8.3 years, 338 participants developed first CVD events. Plasma NGAL was weakly to moderately correlated with several CVD risk markers. There was a non-linear relationship between NGAL and CVD risk. In analyses adjusted for established risk factors, the hazard ratio (95% CI) for CVD in a comparison of the top quartile versus bottom quartiles 1-2 of NGAL values was 1.35 (1.05-1.75; *P*=0.022), which was abrogated after additional adjustment for other potential confounders (mainly attributed to high sensitivity C-reactive protein) 1.20 (0.92-1.57; *P*=0.176). The association was considerably attenuated following further adjustment for renal function 1.05 (0.79-1.40; *P*=0.745). The association between NGAL and CVD risk did not vary importantly in relevant clinical subgroups.

Conclusion: Evidence suggests a non-linear association between NGAL and CVD risk, which is dependent on inflammation and renal function.

Keywords: Neutrophil gelatinase-associated lipocalin; cardiovascular disease; risk factor; cohort study

1. Introduction

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 or oncogene 24p3, is a human protein that is mainly expressed by neutrophils and in low levels by various epithelial cells.[1-3] The physiological functions of NGAL include involvement in the innate immune response to bacterial infection[4] and it also functions as a growth factor.[5] NGAL has emerged as a precise and sensitive novel biomarker for acute kidney injury (AKI);[6, 7] within two hours of an AKI, high levels of NGAL are secreted into the blood and urine.[6] NGAL measurements also have potential relevance for chronic kidney disease (CKD), nephropathy, and kidney transplant.[8, 9] Beyond the kidney, [10]emerging evidence suggests that NGAL may be implicated in the pathogenesis of atherosclerotic CVD. Recent data from both human studies and animal models have demonstrated NGAL to be highly expressed in thrombi and atherosclerotic plaques.[11-14] NGAL is also highly expressed in the heart[13] and elevated levels have been demonstrated in patients with heart failure, coronary heart disease or syndromes, and stroke.[15-17] A number of studies have also reported associations between elevated NGAL levels and poor outcomes (e.g., mortality, hospital re-admissions, heart failure, major adverse cardiac events) in patients with pre-existing CVD or kidney disease.[18-23] The overall evidence suggests that NGAL may be involved in CVD development. However, because the existing studies were (i) mostly cross-sectional evaluations of clinical studies; (ii) conducted in animal models or in patients with pre-existing disease; or (iii) either not sufficiently powered or did not account adequately for potential confounders; the temporal nature of the relationship between circulating NGAL and risk of CVD is not certain. A limited number of population-based prospective studies have however reported associations between increased circulating levels of NGAL and increased risk of first cardiovascular events in the general population.[24, 25] Though these previous studies were elegantly analyzed, they had a number of limitations which included not assessing the nature of the dose-response relationship between circulating NGAL and CVD risk and whether the association is modified by relevant clinical characteristics. In this context, we aimed to investigate in greater detail than ever before, aspects of

the association such as the shape, nature, magnitude, and consistency of the prospective association between plasma NGAL and risk of first-onset CVD events using a population-based cohort of 5,275 participants who were free from pre-existing CVD at baseline.

2. Materials and methods

2.1. Study design and population

We conducted the current study according to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (**Supplementary material 1**).[26] We employed the Prevention of Renal and Vascular End-stage Disease (PREVEND) study for this analysis. PREVEND is a general population-based prospective cohort study which was designed to explore the natural course of urinary albumin excretion (UAE) and its association with kidney disease and CVD. The study design and recruitment processes have been described previously.[27-29] The PREVEND cohort consists of a representative sample of men and women living in the city of Groningen in the Netherlands. The current cohort involved 6894 individuals aged 32-80 years who were invited for the second screening phase of the PREVEND study and who had baseline assessments performed between 2001 and 2003. For this analysis, we included participants without pre-existing CVD, renal disease, or malignancy at baseline. The current analysis involved 5275 participants with non-missing information on plasma NGAL, relevant covariates, and cardiovascular outcomes. The local ethics committee of the University Medical Center Groningen approved the study protocol. The study procedures were conducted according to the Declaration of Helsinki and written informed consent was obtained from all study participants.

2.2. Measurement of NGAL and risk markers

Baseline data on demographics, anthropometric measurements, lifestyle factors, and other cardiovascular risk markers were collected during two outpatient visits by study participants. Venous blood samples were obtained from study participants after an overnight fast and 15 minutes of rest prior to sample collection. Plasma samples were prepared by centrifugation at 4 ºC and samples stored

at -80 ºC until analysis. Plasma NGAL concentrations were measured using Gentian NGAL turbidimetric immunoassay (Gentian, Moss, Norway) applied on a Mindray BS-400 analyser (Mindray, Shenzhen, China). The Gentian NGAL assay was calibrated to the commercially available Bioporto NGAL Test, using the value transfer protocol as described by Blirup-Jensen et al.[30] In addition, the Gentian NGAL calibrator has been validated according to the Clinical and Laboratory Standards Institute guidelines, with external validation performed by individual laboratories.[31] Fasting plasma glucose (FPG) was measured by dry chemistry (Eastman Kodak, Rochester, New York). Total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides, and high sensitivity C-reactive protein (hsCRP) were measured using standard protocols previously described.[32-36] Serum creatinine was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York). Serum cystatin C concentrations were measured by Gentian Cystatin C Immunoassay (Gentian AS, Moss, Norway) on a Modular analyzer (Roche Diagnostics). Cystatin C was calibrated directly using the standard supplied by the manufacturer (traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C).[37] The intra- and inter-assay coefficients of variation were <4.1% and <3.3%, respectively. UAE was estimated as the mean of two 24-hour urine collections and the concentration was determined by nephelometry (BNII; Dade Behring Diagnostic, Marburg, Germany). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation was used to calculate estimated glomerular filtration rate (eGFR).[38] Type 2 diabetes was defined as a FPG level of ≥ 7.0 mmol/l, a nonfasting glucose level of ≥11.1 mmol/l or use of antidiabetic medication according to self-report or to pharmacy data.[39]

2.3. Ascertainment of outcomes

We included first-onset cardiovascular outcomes that occurred from study enrollment through to 01 January 2011. Composite CVD was the primary outcome for this analysis, with CHD and stroke endpoints as subsidiary outcomes. The sources of information on deaths were ascertained by computerized data linkage with the Dutch Central Bureau of Statistics. The Dutch national registry of hospital discharge diagnoses (PRISMANT) was the source of data on cardiovascular morbidity

hospitalizations.[40] Outcome data were coded according to the *International Classification of Diseases*, Ninth Revision (ICD-9) until 01 January 2009; after which ICD-10 codes were used. We defined composite CVD as the combined outcomes of acute and subacute ischemic heart disease (IHD), acute myocardial infarction (AMI), percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG), intracerebral hemorrhage, other intracranial hemorrhage, subarachnoid hemorrhage, stenosis or occlusion of the precerebral or cerebral arteries, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of aorta. Coronary heart disease was defined as fatal or nonfatal IHD, fatal or nonfatal MI, PTCA, and CABG. Stroke events were defined as intracerebral hemorrhage, other intracranial hemorrhage, subarachnoid hemorrhage, stenosis or occlusion of the precerebral or cerebral arteries, and carotid obstruction.

2.4. Statistical analyses

We log-transformed values of skewed variables to achieve approximately symmetrical distributions. Baseline characteristics were presented as means (standard deviation, SD) or median (interquartile range, IQR) for continuous variables and percentages for categorical variables. We calculated partial correlation coefficients (adjusted for age and sex) to evaluate the cross-sectional associations of plasma NGAL levels with CVD risk markers. Cox proportional hazards regression models were used to assess the associations of plasma NGAL with risk of CVD, after confirming no major departure from hazards proportionality assumptions using Schoenfeld residuals.[41] We plotted cumulative Kaplan-Meier curves for CVD during follow-up according to categories of NGAL. We assessed the shape of the association of plasma NGAL with CVD risk by plotting hazard ratios (HRs) calculated within quartiles of baseline NGAL values (relative to the bottom quartile) against the mean NGAL value in each quartile using floating absolute risks (FARs).[42] As the association showed a non-linear shape, NGAL was categorised as fourths defined according to its baseline distribution. Because of the relatively flat risk of CVD across quartiles 1-2, these categories were combined and served as the reference comparison. In a subsidiary analysis, quartile 2 was used as the reference comparison. Hazard ratios were calculated with adjustment for confounders in four models: i) age and sex; ii) smoking status, history of type 2 diabetes, systolic blood pressure (SBP), total cholesterol, and

HDL-C; iii) other potential confounders [triglycerides, body mass index (BMI), alcohol consumption, FPG, and hsCRP]; and iv) UAE and eGFR. We explored if the association is modified by relevant clinical characteristics using tests of interaction. Finally, we conducted sensitivity analyses which employed the use of complex survey design analyses;[43] this is to take into account that the PREVEND cohort is oversampled for subjects with higher albuminuria levels, which enables the results to be extrapolated to the broader (general) population. All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

3. Results

3.1. Baseline characteristics

Baseline characteristics of study participants are presented in **Table 1**. Overall, mean age of participants at study entry was 53 (SD, 12) years and 47.8% were men. There were significant differences in baseline characteristics between participants who did and did not develop CVD. Participants who developed CVD at follow-up were more likely to be older; be smokers; have a history of type 2 diabetes; be using antihypertensive and lipid-lowering medications; have higher BMI and blood pressure; have higher circulating NGAL, total cholesterol, triglycerides and lower HDL-C levels; have higher levels of markers of inflammation and metabolic function; and have lower renal function at study entry compared with those who did not develop CVD. The mean (SD) plasma concentration of NGAL was 105.8 (37.2) µg/L. NGAL was weakly correlated with several risk markers: positively with age, triglycerides, hsCRP, creatinine, and UAE; and inversely with HDL-C and FPG. Moderately strong correlations were observed for cystatin C (r=0.41) and eGFR (r=-0.34). Baseline NGAL concentrations were higher by 5.65% in males compared with females, by 13.49% in current smokers compared with never and former smokers, and by 5.32% in subjects on antihypertensive medication compared with non-users. The levels were lower by 4.07% in subjects with type 2 diabetes compared to those without type 2 diabetes (**Table 2**).

3.2. Plasma NGAL and risk of incident CVD

A total of 338 incident CVD events (annual rate 8.21/1,000 person-years at risk; 95% CI: 7.38 to 9.14) were recorded during a median follow-up of 8.3 (IQR, 7.7-8.9) years. Cumulative hazard curves showed an increased risk of CVD in the top quartile of NGAL levels compared with quartiles 1-2 and 3 (*P*-value for log-rank test < 0.001 for all; **Figure 1**). In analyses adjusted for age and sex and also for conventional risk factors, a non-linear relationship was observed between NGAL and CVD risk (**Figure 2**). **Table 3** reports the associations of plasma NGAL with the risk of CVD outcomes in analyses that compared the top quartile versus bottom quartiles 1-2 of the NGAL distribution. The age- and sex-adjusted HR for CVD was 1.48 (95% CI: 1.15 to 1.91), which was minimally attenuated to 1.35 (95% CI: 1.05 to 1.75) following further adjustment for conventional CVD risk factors. However, further adjustment for $\log_e$ triglycerides, BMI, alcohol consumption, FPG, and $\log_e$ hsCRP attenuated the association to 1.20 (95% CI: 0.92 to 1.57). The non-significant association was considerably attenuated following further adjustment for $\log_e$ UAE and eGFR 1.05 (95% CI: 0.79 to 1.40). In an age- and sex-adjusted analysis, the NGAL-CVD association was abrogated after single additional adjustment for $\log_e$ hsCRP 1.21 (95% CI: 0.93 to 1.57) and eGFR 1.26 (95% CI: 0.96 to 1.66). The associations were non-significant for CHD and stroke (**Table 3**). All results were essentially similar when design-based Cox regression analyses were conducted (**Table 4**). In subsidiary analysis that compared the top quartile versus quartile 2 of the NGAL distribution, the associations were qualitatively similar (**Table 5**). The association between NGAL and CVD risk did not vary significantly by levels or categories of several clinically relevant characteristics and other risk markers (*P* for interaction ≥ 0.10 for each; **Figure 3**).

4. Discussion

4.1. Main findings

In this large-scale population-based study of Caucasian males and females without a history of CVD at study entry, baseline NGAL levels were generally weakly correlated with several cardiovascular risk markers. Moderately strong correlations were observed with measures of renal function. Characterization of the shape of the association showed a non-linear relationship between

NGAL and CVD risk. The association which was independent of conventional risk factors and other potential confounders, was attenuated on accounting for inflammation and renal function. The association was not modified by clinically relevant subgroups and remained persistent in analyses that took into account that the PREVEND cohort is oversampled for subjects with higher albuminuria levels. There was no evidence of associations of NGAL with CHD or stroke endpoints; findings which might be attributed to the low event rates. Further work is required to explore the associations of NGAL with these outcomes.

4.2. Comparison with previous work

In 1,393 community-dwelling older adults of the Rancho Bernardo Study (RBS) who were followed for an average period of 11 years, Daniels and colleagues demonstrated NGAL to be independently associated CVD mortality as well as composite CVD. The association was also independent of renal function (as measured by creatinine clearance), N-terminal pro B-type natriuretic peptide (NT-proBNP), and CRP.[25] NGAL was also demonstrated to add prognostic value on top of established cardiovascular risk factors and biomarkers such as NT-proBNP and CRP. Lindberg and colleagues also reported similar findings in the fourth Copenhagen Heart Study (CHS) of 5,599 community dwelling men and women followed for an average period of 10 years.[24] Unlike findings of these previous studies which were also based in general population settings, our results show that the association between NGAL and CVD risk is dependent on inflammation as well as renal function. The inconsistencies in the findings could be related to differences in sample sizes; population characteristics such as age or presence of undiagnosed pre-existing disease at baseline; sample storage and assay methods for NGAL measurements; distribution of NGAL; or outcomes. For example, though the sample sizes and age distribution of participants for the PREVEND cohort and fourth CHS were similar, the RBS was based on a relatively smaller sample size of elderly participants. The stronger and independent associations demonstrated in RBS participants could be a consequence of selection bias, associated with the inclusion of individuals who may already be at increased risk of CVD. Indeed, stronger associations have been observed between NGAL and cardiovascular mortality in individuals with pre-existing CVD.[44] NGAL was normally distributed in the PREVEND cohort,

whiles the distribution was skewed in the RBS and fourth CHS cohorts.[24, 25] Since plasma NGAL levels in a healthy population mainly reflects inflammation,[24] it is possible that the skewed distribution of plasma NGAL in these cohorts could reflect an over-inclusion of participants with low-grade inflammation, hence the stronger associations which were independent of inflammation. Though previous studies also involved prolonged sample storage at -70 or -80 °C, there is a possibility that storage as well as freeze/thawing methods in the PREVEND study could have contributed to the weak associations demonstrated in our analyses due to sample degradation. Evidence on whether plasma NGAL is influenced by prolonged storage for several years is sparse; however, plasma NGAL has been shown to be stable using repeated freeze-thaw cycles and can remain stable for at least 11 months at -80 °C.[45, 46] In the PREVEND study, assay methods employed a particle-enhanced turbidimetric immunoassay for NGAL not calibrated to the commercial available standardized point-of-care assay – Triage® NGAL test – which provides non-biased measurements of plasma NGAL.[1] The fourth CHS used an in-house time resolved immunofluorometric assay based on NGAL antibodies, whiles the RBS used a competitive immunoassay developed on the Luminex platform, which was also not calibrated to the commercial available assay. Finally, there was a difference in outcome between the PREVEND cohort and fourth CHS. The fourth CHS evaluated major adverse cardiovascular events which comprised of mortality or hospitalization due to AMI, ischemic stroke or heart failure; however, the PREVEND study did not include heart failure in the CVD outcome definition, but in addition included IHD, PTCA or CABG, intracerebral hemorrhage, other intracranial hemorrhage, subarachnoid hemorrhage, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of aorta.

Our findings also provide new observations previously not reported in the general population setting – we showed a non-linear shape to the association and therefore appropriately modelled NGAL as a categorical variable compared to previous studies that assumed a linear relationship to the association. Consistent with our findings, in a prospective evaluation of patients undergoing coronary angiography in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, a U-shaped relationship was demonstrated between NGAL and mortality (cardiovascular and all-cause mortality).[23] Furthermore in their findings, the association was abrogated on further adjustment for

eGFR. Interestingly, the LURIC study employed NGAL assay methods which were similar to that of the PREVEND study. Finally, in a comprehensive subgroup analyses, we also demonstrated that the NGAL-CVD association was not modified by relevant clinical characteristics such as age, sex, levels or categories of other cardiovascular risk factors, inflammation, and renal function.

4.3. Possible explanations for findings

NGAL has multiple functions beyond its well-known property as a marker of renal function. Accumulating evidence provides biological plausibility for the potential role of NGAL in CVD development. Evidence from both human studies and animal models have shown NGAL to be highly expressed in thrombi and atherosclerotic plaques,[11-14] suggesting that NGAL may be involved in the atherosclerotic process. NGAL is highly expressed in the heart with suggestion of a potential role in the pathogenesis of myocarditis.[47] NGAL has been demonstrated to co-localize with matrix metalloproteinase (MMP)-9 in the cytosol and lipid core of atherosclerotic plaques;[10, 11, 14] MMP-9 is an important mediator of atherosclerosis, plaque instability and vascular remodeling.[48, 49] Evidence suggests the presence of NGAL inhibits the degradation of MMP-9 resulting in the preservation of its enzymatic activity[50] and the NGAL-MMP-9 interaction is relevant to the development of atherosclerotic plaques and CHD.[51] NGAL mediates inflammatory response[52] and is upregulated in inflammatory vascular damage,[53] suggesting its role in atherosclerotic CVD via inflammatory processes. Indeed, the association between NGAL and CVD was abrogated on further adjustment for hsCRP, lending further support to the inflammation hypothesis of atherosclerotic CHD development. NGAL is also upregulated in endothelial dysfunction, which plays an important role in CVD development.[54] Whether NGAL plays a direct role in the etiology of CVD or may just be a marker of underlying pathology is uncertain. Further clarification of the role of NGAL in the pathogenesis of CVD is required in mechanistic studies.

4.4. Implications of findings

Irrespective of the dependence on the association between NGAL and CVD risk on inflammation and renal function; the current findings are relevant and may have implications for CVD prevention.

Findings suggest that elevated circulating levels are associated with an increased risk of future CVD. Whether NGAL is just a risk marker for CVD, an emerging cardiovascular risk factor, or causal therapeutic target, remains to be ascertained. However, the current findings suggest that NGAL may be a risk marker for CVD. In the absence of evidence establishing a potential relevance of circulating NGAL in CVD prevention strategies, individuals with elevated NGAL levels may need clinical evaluation of their cardiovascular risk.

4.5. Strengths and limitations

The notable strengths of the current study include the large sample size of participants who were identified from population registers and were representative of the general population; the follow-up for ascertainment of outcomes was long; there was information on a comprehensive panel of lifestyle and biological markers which enabled adequate adjustment for relevant potential confounding; the design minimized possibilities of reverse causation bias as it involved individuals free of vascular disease or malignant disorders at baseline; and the analysis was comprehensive which included characterization of the shape of the association, evaluation of effect modification, and sensitivity analyses. The limitations of the current study also deserve consideration. As with observational cohort designs, there was potential for residual confounding due to errors in confounder measurements and/or other unmeasured covariates. The findings were demonstrated in white European Caucasians and therefore cannot be generalized to other ethnicities. NGAL was measured at only one point in time, which precluded the ability to correct for regression dilution bias and this could have underestimated the associations demonstrated. We had no data on urine NGAL which precluded assessment of its associations with CVD as well as comparisons with plasma NGAL. Non-biased measurements of urine NGAL are obtained using the standardized ARCHITECT immunoassay platform (ARCHITECT® analyzer, Abbot Diagnostics) and are also not cumbersome to perform just like the Triage® NGAL assay for plasma NGAL. However, urine NGAL measurements require more time (within 35 min) compared to plasma NGAL (15 min).[1] Given that circulating NGAL may be mainly derived from activated neutrophils whiles urine NGAL may be tubular epithelial cells derived,[44] it has been suggested that urine NGAL may be a more robust indicator of AKI compared

with plasma NGAL. Whether urine or plasma NGAL is a more robust indicator of CVD risk is uncertain, as there is limited evidence on comparative studies. In a cohort of 597 Swedish men aged 78 years who were followed up for a median period of 8.1 years, urine NGAL was shown to be associated with CVD and all-cause mortality independently of cardiovascular risk factors including eGFR and CRP; however, serum NGAL was not independently associated with both outcomes in the same study population.[44] In this same study, no significant correlation was demonstrated between serum and urine NGAL. The authors suggest that these findings might reflect the different origins of serum and urine NGAL. Daniels and colleagues in their report of the RBS indicated that the potential role of plasma NGAL in the development of CVD may be the distinguishing characteristic between plasma NGAL and urine NGAL.[25] However, in their analyses, no comparisons were made with urine NGAL. Among 3,386 patients with CKD, urine NGAL was shown to be independently associated with the risk of future ischemic atherosclerotic events, but not with heart failure or all-cause mortality.[55] In another study of 2,466 patients with CKD, among which 34% had a self-reported history of any CVD, urine NGAL was associated with all-cause mortality but not with heart failure or atherosclerotic CVD.[22] Further large-scale studies are needed to robustly compare the associations of plasma and urine NGAL with cardiovascular outcomes and evaluate their potential utility in predicting risk.

In conclusion, in a predominantly Caucasian population, plasma NGAL is associated with an increased risk of CVD in a non-linear fashion. The association is independent of established risk factors but is dependent on inflammation and renal function.

Conflicts of interest

Tom Nilsen, Clara Hidden, and Erling Sundrehagen are employees of the manufacturer of the NGAL assay, Gentian, in Moss, Norway.

Authorship

SKK researched data, analyzed data and wrote the manuscript. SKK, JLF-G, LMK, TN, CH, ES, SS, RPFD, and SJLB contributed to data collection, reviewed and edited the manuscript. SJLB

supervised the study and SKK is the guarantor and had full access to all the data in the study and takes responsibility for the integrity of the data and the decision to submit and publish the manuscript.

Ethics

This study protocol was evaluated by the ethical committee of the University Medical Center Groningen. A written consent was obtained from each patient evaluated in this study.

Funding

The Dutch Kidney Foundation supported the infrastructure of the PREVEND program from 1997 to 2003 (Grant E.033). The University Medical Center Groningen supported the infrastructure from 2003 to 2006. Dade Behring, Ausam, Roche, and Abbott financed laboratory equipment and reagents by which various laboratory determinations could be performed. The Dutch Heart Foundation supported studies on lipid metabolism (Grant 2001-005). The contents of this paper are solely the responsibility of the authors and do not represent the views of the Sponsors. The funder had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Dr. Setor K. Kunutsor acknowledges support from the NIHR Biomedical Research Centre at University Hospitals Bristol NHS Foundation Trust and the University of Bristol. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health and Social Care. NGAL concentrations were determined by Gentian, Moss, Norway.

References

[1] P. Devarajan, Neutrophil gelatinase-associated lipocalin--an emerging troponin for kidney injury, Nephrol Dial Transplant 23(12) (2008) 3737-43.

[2] J.B. Cowland, N. Borregaard, Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans, Genomics 45(1) (1997) 17-23.

[3] A. Friedl, S.P. Stoesz, P. Buckley, M.N. Gould, Neutrophil gelatinase-associated lipocalin in normal and neoplastic human tissues. Cell type-specific pattern of expression, Histochem J 31(7) (1999) 433-41.

[4] D.H. Goetz, M.A. Holmes, N. Borregaard, M.E. Bluhm, K.N. Raymond, R.K. Strong, The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition, Mol Cell 10(5) (2002) 1033-43.

[5] K.M. Schmidt-Ott, K. Mori, J.Y. Li, A. Kalandadze, D.J. Cohen, P. Devarajan, J. Barasch, Dual action of neutrophil gelatinase-associated lipocalin, J Am Soc Nephrol 18(2) (2007) 407-13.

[6] M. Bennett, C.L. Dent, Q. Ma, S. Dastrala, F. Grenier, R. Workman, H. Syed, S. Ali, J. Barasch, P. Devarajan, Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study, Clin J Am Soc Nephrol 3(3) (2008) 665-73.

[7] W.K. Han, G. Wagener, Y. Zhu, S. Wang, H.T. Lee, Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery, Clin J Am Soc Nephrol 4(5) (2009) 873-82.

[8] S.L. Goldstein, Acute kidney injury biomarkers: renal angina and the need for a renal troponin I, BMC Med 9 (2011) 135.

[9] S.S. Soni, D. Cruz, I. Bobek, C.Y. Chionh, F. Nalesso, P. Lentini, M. de Cal, V. Corradi, G. Virzi, C. Ronco, NGAL: a biomarker of acute kidney injury and other systemic conditions, Int Urol Nephrol 42(1) (2010) 141-50.

[10] M. Folkesson, M. Kazi, C. Zhu, A. Silveira, A.L. Hemdahl, A. Hamsten, U. Hedin, J. Swedenborg, P. Eriksson, Presence of NGAL/MMP-9 complexes in human abdominal aortic aneurysms, Thromb Haemost 98(2) (2007) 427-33.

[11] A.L. Hemdahl, A. Gabrielsen, C. Zhu, P. Eriksson, U. Hedin, J. Kastrup, P. Thoren, G.K. Hansson, Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction, Arterioscler Thromb Vasc Biol 26(1) (2006) 136-42.

[12] C. Tarin, C.E. Fernandez-Garcia, E. Burillo, C. Pastor-Vargas, P. Llamas-Granda, B. Castejon, P. Ramos-Mozo, M.M. Torres-Fonseca, T. Berger, T.W. Mak, J. Egido, L.M. Blanco-Colio, J.L. Martin-Ventura, Lipocalin-2 deficiency or blockade protects against aortic abdominal aneurysm development in mice, Cardiovasc Res 111(3) (2016) 262-73.

[13] A. Yndestad, L. Landro, T. Ueland, C.P. Dahl, T.H. Flo, L.E. Vinge, T. Espevik, S.S. Froland, C. Husberg, G. Christensen, K. Dickstein, J. Kjekshus, E. Oie, L. Gullestad, P. Aukrust, Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure, Eur Heart J 30(10) (2009) 1229-36.

[14] B.C. te Boekhorst, S.M. Bovens, W.E. Hellings, P.H. van der Kraak, K.W. van de Kolk, A. Vink, F.L. Moll, M.F. van Oosterhout, J.P. de Vries, P.A. Doevendans, M.J. Goumans, D.P. de Kleijn, C.J. van Echteld, G. Pasterkamp, J.P. Sluijter, Molecular MRI of murine atherosclerotic plaque targeting

NGAL: a protein associated with unstable human plaque characteristics, Cardiovasc Res 89(3) (2011) 680-8.

[15] K. Damman, D.J. van Veldhuisen, G. Navis, A.A. Voors, H.L. Hillege, Urinary neutrophil gelatinase associated lipocalin (NGAL), a marker of tubular damage, is increased in patients with chronic heart failure, Eur J Heart Fail 10(10) (2008) 997-1000.

[16] T. Zografos, A. Haliassos, S. Korovesis, E. Giazitzoglou, E. Voridis, D. Katritsis, Association of neutrophil gelatinase-associated lipocalin with the severity of coronary artery disease, Am J Cardiol 104(7) (2009) 917-20.

[17] A.M. Elneihoum, P. Falke, L. Axelsson, E. Lundberg, F. Lindgarde, K. Ohlsson, Leukocyte activation detected by increased plasma levels of inflammatory mediators in patients with ischemic cerebrovascular diseases, Stroke 27(10) (1996) 1734-8.

[18] A.B. Akcay, M.F. Ozlu, N. Sen, S. Cay, O.H. Ozturk, F. Yalcn, P. Bilen, S. Kanat, M.F. Karakas, A. Isleyen, A.D. Demir, S. Sogut, A. Covic, M. Kanbay, Prognostic significance of neutrophil gelatinase-associated lipocalin in ST-segment elevation myocardial infarction, J Investig Med 60(2) (2012) 508-13.

[19] S. Lindberg, S.H. Pedersen, R. Mogelvang, J.S. Jensen, A. Flyvbjerg, S. Galatius, N.E. Magnusson, Prognostic utility of neutrophil gelatinase-associated lipocalin in predicting mortality and cardiovascular events in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention, J Am Coll Cardiol 60(4) (2012) 339-45.

[20] M.V. Zykov, V.V. Kashtalap, I.S. Bykova, O.N. Hryachkova, V.V. Kalaeva, K.S. Shafranskaya, V.N. Karetnikova, O.L. Barbarash, [Clinical and Prognostic Value of Serum Neutrophil Gelatinase-Associated Lipocalin in Patients With ST-Segment Elevation Myocardial Infarction], Kardiologiia 56(5) (2016) 24-29.

[21] V. Karetnikova, A. Osokina, O. Gruzdeva, E. Uchasova, M. Zykov, V. Kalaeva, V. Kashtalap, K. Shafranskaya, O. Barbarash, Serum neutrophil gelatinase-associated lipocalin the estimation of hospital prognosis in patients with ST-elevated myocardial infarction, PloS one 12(7) (2017) e0180816.

[22] M. Park, C.Y. Hsu, A.S. Go, H.I. Feldman, D. Xie, X. Zhang, T. Mifflin, S.S. Waikar, V.S. Sabbisetti, J.V. Bonventre, J. Coresh, R.G. Nelson, P.L. Kimmel, J.W. Kusek, M. Rahman, J.R. Schelling, R.S. Vasan, K.D. Liu, I. Chronic Renal Insufficiency Cohort Study, C.K.D.B. Consortium, Urine Kidney Injury Biomarkers and Risks of Cardiovascular Disease Events and All-Cause Death: The CRIC Study, Clin J Am Soc Nephrol 12(5) (2017) 761-771.

[23] R.P. Woitas, H. Scharnagl, M.E. Kleber, G.E. Delgado, T.B. Grammer, M. Pichler, B.K. Kramer, W. Marz, T. Stojakovic, Neutrophil gelatinase-associated lipocalin levels are U-shaped in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study-Impact for mortality, PloS one 12(2) (2017) e0171574.

[24] S. Lindberg, J.S. Jensen, R. Mogelvang, S.H. Pedersen, S. Galatius, A. Flyvbjerg, N.E. Magnusson, Plasma neutrophil gelatinase-associated lipocalinin in the general population: association with inflammation and prognosis, Arterioscler Thromb Vasc Biol 34(9) (2014) 2135-42.

[25] L.B. Daniels, E. Barrett-Connor, P. Clopton, G.A. Laughlin, J.H. Ix, A.S. Maisel, Plasma neutrophil gelatinase-associated lipocalin is independently associated with cardiovascular disease and mortality in community-dwelling older adults: The Rancho Bernardo Study, J Am Coll Cardiol 59(12) (2012) 1101-9.

[26] E. von Elm, D.G. Altman, M. Egger, S.J. Pocock, P.C. Gotzsche, J.P. Vandenbroucke, The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies, Journal of clinical epidemiology 61(4) (2008) 344-9.

[27] S.K. Kunutsor, S.J. Bakker, J.E. Kootstra-Ros, R.T. Gansevoort, R.P. Dullaart, Circulating gamma glutamyltransferase and prediction of cardiovascular disease, Atherosclerosis 238(2) (2014) 356-364.

[28] S.K. Kunutsor, L.M. Kieneker, S.J.L. Bakker, R.W. James, R.P.F. Dullaart, Incident type 2 diabetes is associated with HDL, but not with its anti-oxidant constituent - paraoxonase-1: The prospective cohort PREVEND study, Metabolism - Clinical and Experimental (2017).

[29] S.K. Kunutsor, L.M. Kieneker, S. Burgess, S.J.L. Bakker, R.P.F. Dullaart, Circulating Total Bilirubin and Future Risk of Hypertension in the General Population: The Prevention of Renal and Vascular End-Stage Disease (PREVEND) Prospective Study and a Mendelian Randomization Approach, Journal of the American Heart Association 6(11) (2017).

[30] S. Blirup-Jensen, A.M. Johnson, M. Larsen, I.C.o.P. Proteins, Protein standardization V: value transfer. A practical protocol for the assignment of serum protein values from a Reference Material to a Target Material, Clin Chem Lab Med 46(10) (2008) 1470-9.

[31] G.L. Salvagno, A. Ferrari, M. Gelati, G. Brocco, G. Lippi, Analytical validation of Gentian NGAL particle-enhanced enhanced turbidimetric immunoassay (PETIA), Pract Lab Med 8 (2017) 60-64.

[32] S.E. Borggreve, H.L. Hillege, G.M. Dallinga-Thie, P.E. de Jong, B.H. Wolffenbuttel, D.E. Grobbee, A. van Tol, R.P. Dullaart, P.S. Group, High plasma cholesteryl ester transfer protein levels may favour reduced incidence of cardiovascular events in men with low triglycerides, Eur Heart J 28(8) (2007) 1012-8.

[33] R.P. Dullaart, F. Perton, M.M. van der Klauw, H.L. Hillege, W.J. Sluiter, P.S. Group, High plasma lecithin:cholesterol acyltransferase activity does not predict low incidence of cardiovascular events: possible attenuation of cardioprotection associated with high HDL cholesterol, Atherosclerosis 208(2) (2010) 537-42.

[34] J.P. Corsetti, S.J. Bakker, C.E. Sparks, R.P. Dullaart, Apolipoprotein A-II influences apolipoprotein E-linked cardiovascular disease risk in women with high levels of HDL cholesterol and C-reactive protein, PloS one 7(6) (2012) e39110.

[35] S.K. Kunutsor, S.J. Bakker, J.E. Kootstra-Ros, H. Blokzijl, R.T. Gansevoort, R.P. Dullaart, Inverse linear associations between liver aminotransferases and incident cardiovascular disease risk: The PREVEND study, Atherosclerosis 243(1) (2015) 138-47.

[36] S.K. Kunutsor, S.J. Bakker, J.E. Kootstra-Ros, R.T. Gansevoort, J. Gregson, R.P. Dullaart, Serum Alkaline Phosphatase and Risk of Incident Cardiovascular Disease: Interrelationship with High Sensitivity C-Reactive Protein, PloS one 10(7) (2015) e0132822.

[37] A. Grubb, S. Blirup-Jensen, V. Lindstrom, C. Schmidt, H. Althaus, I. Zegers, I.W.G.o.S.o.C. C, First certified reference material for cystatin C in human serum ERM-DA471/IFCC, Clin Chem Lab Med 48(11) (2010) 1619-21.

[38] L.A. Inker, C.H. Schmid, H. Tighiouart, J.H. Eckfeldt, H.I. Feldman, T. Greene, J.W. Kusek, J. Manzi, F. Van Lente, Y.L. Zhang, J. Coresh, A.S. Levey, C.-E. Investigators, Estimating glomerular filtration rate from serum creatinine and cystatin C, N Engl J Med 367(1) (2012) 20-9.

[39] A. Abbasi, E. Corpeleijn, R.T. Gansevoort, R.O. Gans, H.L. Hillege, R.P. Stolk, G. Navis, S.J. Bakker, R.P. Dullaart, Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: the PREVEND study, J Clin Endocrinol Metab 98(8) (2013) E1352-9.

[40] B.H. Stricker, R.M. Herings, [Plea for the retention of the Dutch National Medical Registration (LMR) to provide reliable information regarding public health and healthcare], Nederlands tijdschrift voor geneeskunde 150(35) (2006) 1916-7.

[41] T.M. Therneau, P.M. Grambsch, Modeling Survival Data: Extending the Cox Model, Springer, New York, NY, 2000, pp. 39-77.

[42] D.F. Easton, J. Peto, A.G. Babiker, Floating absolute risk: an alternative to relative risk in survival and case-control analysis avoiding an arbitrary reference group, Stat Med 10(7) (1991) 1025-35.

[43] S.G. Heeringa, B.T. West, P.A. Berglund, Applied Survey Data Analysis, Chapman & Hall/CRC2010.

[44] J. Helmersson-Karlqvist, A. Larsson, A.C. Carlsson, P. Venge, J. Sundstrom, E. Ingelsson, L. Lind, J. Arnlov, Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with mortality in a community-based cohort of older Swedish men, Atherosclerosis 227(2) (2013) 408-13.

[45] K.R. Pedersen, H.B. Ravn, V.E. Hjortdal, R. Norregaard, J.V. Povlsen, Neutrophil gelatinase-associated lipocalin (NGAL): validation of commercially available ELISA, Scand J Clin Lab Invest 70(5) (2010) 374-82.

[46] N.E. Magnusson, M. Hornum, K.A. Jorgensen, J.M. Hansen, C. Bistrup, B. Feldt-Rasmussen, A. Flyvbjerg, Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation, BMC Nephrol 13 (2012) 8.

[47] L. Ding, H. Hanawa, Y. Ota, G. Hasegawa, K. Hao, F. Asami, R. Watanabe, T. Yoshida, K. Toba, K. Yoshida, M. Ogura, M. Kodama, Y. Aizawa, Lipocalin-2/neutrophil gelatinase-B associated lipocalin is strongly induced in hearts of rats with autoimmune myocarditis and in human myocarditis, Circ J 74(3) (2010) 523-30.

[48] D.L. Brown, M.S. Hibbs, M. Kearney, C. Loushin, J.M. Isner, Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina, Circulation 91(8) (1995) 2125-31.

[49] Z.S. Galis, J.J. Khatri, Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly, Circ Res 90(3) (2002) 251-62.

[50] L. Yan, N. Borregaard, L. Kjeldsen, M.A. Moses, The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL). Modulation of MMP-9 activity by NGAL, J Biol Chem 276(40) (2001) 37258-65.

[51] D.N. Cruz, S. Gaiao, A. Maisel, C. Ronco, P. Devarajan, Neutrophil gelatinase-associated lipocalin as a biomarker of cardiovascular disease: a systematic review, Clin Chem Lab Med 50(9) (2012) 1533-45.

[52] D.H. Goetz, S.T. Willie, R.S. Armen, T. Bratt, N. Borregaard, R.K. Strong, Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin, Biochemistry 39(8) (2000) 1935-41.

[53] D.X. Bu, A.L. Hemdahl, A. Gabrielsen, J. Fuxe, C. Zhu, P. Eriksson, Z.Q. Yan, Induction of neutrophil gelatinase-associated lipocalin in vascular injury via activation of nuclear factor-kappaB, Am J Pathol 169(6) (2006) 2245-53.

[54] J. Barthelmes, M.P. Nagele, V. Ludovici, F. Ruschitzka, I. Sudano, A.J. Flammer, Endothelial dysfunction in cardiovascular disease and Flammer syndrome-similarities and differences, EPMA J 8(2) (2017) 99-109.

[55] K.D. Liu, W. Yang, A.S. Go, A.H. Anderson, H.I. Feldman, M.J. Fischer, J. He, R.R. Kallem, J.W. Kusek, S.R. Master, E.R. Miller, 3rd, S.E. Rosas, S. Steigerwalt, K. Tao, M.R. Weir, C.Y. Hsu, C.S. Investigators, Urine neutrophil gelatinase-associated lipocalin and risk of cardiovascular disease and death in CKD: results from the Chronic Renal Insufficiency Cohort (CRIC) Study, Am J Kidney Dis 65(2) (2015) 267-74.

Table 1. Baseline participant characteristics overall and according to cardiovascular disease development

	Overall (N=5275) Mean (SD) or median (IQR) or n (%)	**Without CVD (N=4937) Mean (SD) median (IQR) or n (%)**	**With CVD (N=338) Mean (SD) or median (IQR) or n (%)**	***P*-value**
Plasma NGAL (µg/L)	105.8 (37.2)	104.9 (35.7)	118.5 (52.5)	< 0.001
Questionnaire				
Male	2519 (47.8)	2278 (46.1)	241 (71.3)	< 0.001
Age at survey (years)	53 (12)	52 (12)	63 (11)	< 0.001
History of type 2 diabetes	281 (5.3)	239 (4.8)	42 (12.4)	< 0.001
Current smokers	1448 (27.5)	1337 (27.1)	111 (32.8)	< 0.001
Alcohol consumers	3988 (75.6)	3756 (76.1)	232 (68.6)	0.002
Use of anti-hypertensive medication	770 (15.5)	658 (14.2)	112 (34.2)	< 0.001
Use of lipid-lowering medication	125 (2.9)	104 (2.6)	21 (7.0)	< 0.001
Physical measurements				
BMI (kg/m^2)	26.5 (4.3)	26.4 (4.3)	27.7 (3.9)	< 0.001
SBP (mmHg)	125 (18)	124 (18)	140 (20)	< 0.001
DBP (mmHg)	73 (9)	73 (9)	79 (9)	< 0.001
Lipid markers				
Total cholesterol (mmol/l)	5.47 (1.04)	5.45 (1.04)	5.77 (1.10)	< 0.001
HDL-C (mmol/l)	1.27 (0.31)	1.28 (0.31)	1.16 (0.29)	< 0.001
Triglycerides (mmol/l)	1.11 (0.80-1.59)	1.09 (0.79-1.57)	1.33 (0.98-1.87)	< 0.001
Metabolic, inflammatory, and renal function markers				
hsCRP (mg/l)	1.30 (0.61-2.89)	1.25 (0.59-2.79)	2.24 (1.05-4.73)	< 0.001
Fasting plasma glucose (mmol/l)	5.00 (1.10)	4.97 (1.07)	5.37 (1.42)	<0.001
Creatinine (µmol/l)	71.0 (62.0-80.0)	71.0 (62.0-79.0)	76.0 (67.0-89.0)	< 0.001
Cystatin C (mg/l)	8.99 (1.99)	8.89 (1.82)	10.39 (3.39)	< 0.001
eGFR (ml/min/1.73 m^2)	85 (10)	85 (9)	77 (15)	< 0.001
eGFR < 60 ml/min/1.73 m^2*	198 (3.9)	147 (3.0)	51 (15.1)	<0.001
eGFR < 90 ml/min/1.73 m^2*	2131 (40.4)	1907 (38.6)	224 (66.3)	< 0.001
UAE (mg/24 hours)	8.43 (6.00-14.89)	8.27 (5.94-14.22)	13.29 (7.82-36.21)	< 0.001

Continuous variables are reported as mean ± SD or median (interquartile range) and categorical variables are reported as n (%); *, reported as n (%); BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; IQR, interquartile range; NGAL, neutrophil gelatinase-associated lipocalin; SBP, systolic blood pressure; SD, standard deviation; UAE, urinary albumin secretion

Table 2. Cross-sectional correlates of plasma NGAL

	Partial correlation r (95% CI)†	**Percentage difference (95% CI) in NGAL levels per 1 SD higher or compared to reference category of correlate‡**
Plasma NGAL (µg/L)	-	-
Sex		
Female	-	Ref
Male	-	5.65% (3.65, 7.65)***
Questionnaire		
Age at survey (years)	0.05 (0.03, 0.08)***	2.01% (1.01, 3.01)***
History of type 2 diabetes		
No	-	Ref
Yes	-	-4.07% (-8.59, 0.46)
Smoking status		
Never and former smokers	-	Ref
Current smokers	-	13.49% (11.27, 15.71)***
Alcohol consumption		
Non-consumers	-	Ref
Current consumers	-	-0.94% (-3.31, 1.43)
Use of anti-hypertensive medication		
No	-	Ref
Yes	-	5.32% (2.29, 8.35)**
Use of lipid-lowering medication		
No		Ref
Yes		3.07% (-3.62, 9.76)
Physical measurements		
BMI (kg/m^2)	0.00 (-0.02, 0.03)	0.13% (-0.90, 1.15)
SBP (mmHg)	0.01 (-0.02, 0.04)	0.41% (-0.74, 1.57)
DBP (mmHg)	-0.02 (-0.05, 0.01)	-0.83% (-1.94, 0.27)
Lipid markers		
Total cholesterol (mmol/l)	-0.03 (-0.06, -0.01)*	-1.31% (-2.34, -0.29)*
HDL-C (mmol/l)	-0.10 (-0.13, -0.08)***	-4.24% (-5.34, -3.15)***
Triglycerides (mmol/l)	0.04 (0.02, 0.07)***	1.63% (0.62, 2.64)*
Metabolic, inflammatory, and renal function markers		
hsCRP (mg/l)	0.29 (0.26, 0.31)***	11.05% (10.07, 12.04)***
Fasting plasma glucose (mmol/l)	-0.07 (-0.09, -0.04)*	-2.54% (-3.57, -1.51)***
Creatinine (µmol/l)	0.26 (0.24, 0.29)***	11.47% (10.33, 12.62)***
Cystatin C (mg/l)	0.41 (0.38, 0.43)***	16.87% (15.85, 17.90)***
eGFR ($ml/min/1.73\ m^2$)	-0.34 (-0.37, -0.32)***	-15.33% (-16.47, -14.19)***
Log_e UAE (mg/24 hours)	0.12 (0.10, 0.15)***	4.74% (3.71, 5.78)***

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; NGAL, neutrophil gelatinase-associated lipocalin; Ref, reference; SD, standard deviation; SBP, systolic blood pressure; UAE, urinary albumin secretion

Asterisks indicate the level of statistical significance: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †Partial correlation coefficients between NGAL and the row variables; ‡Percentage change in NGAL levels per 1 SD increase in the row variable (or for categorical variables, the percentage difference in mean NGAL levels for the category versus the reference) adjusted for age and sex.

Table 3. Prospective association of NGAL with risk of cardiovascular disease outcomes

NGAL	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Cardiovascular disease									
Q1 – Q2	131 / 2639	ref		ref		ref		ref	
Q3	89 / 1319	1.30 (0.99 to 1.71)	0.055	1.24 (0.95 to 1.63)	0.118	1.18 (0.90 to 1.56)	0.229	1.13 (0.85 to 1.49)	0.400
Q4	118 / 1317	1.48 (1.15 to 1.91)	0.002	1.35 (1.05 to 1.75)	0.022	1.20 (0.92 to 1.57)	0.176	1.05 (0.79 to 1.40)	0.745
Coronary heart disease									
Q1 – Q2	99 / 2639	ref		ref		ref		ref	
Q3	66 / 1319	1.27 (0.93 to 1.74)	0.130	1.24 (0.90 to 1.70)	0.181	1.21 (0.88 to 1.67)	0.236	1.16 (0.84 to 1.60)	0.369
Q4	80 / 1317	1.32 (0.98 to 1.78)	0.067	1.20 (0.89 to 1.63)	0.234	1.12 (0.82 to 1.54)	0.473	0.99 (0.70 to 1.39)	0.938
Stroke									
Q1 – Q2	36 / 2639	ref		ref		ref		ref	
Q3	21 / 1319	1.14 (0.66 to 1.95)	0.641	1.07 (0.62 to 1.84)	0.819	0.98 (0.57 to 1.70)	0.948	0.95 (0.54 to 1.66)	0.854
Q4	34 / 1317	1.56 (0.97 to 2.52)	0.067	1.48 (0.91 to 2.40)	0.114	1.17 (0.71 to 1.94)	0.536	1.07 (0.62 to 1.86)	0.799

CI, confidence interval; HR, hazard ratio; NGAL, neutrophil gelatinase-associated lipocalin
Model 1: Age and sex
Model 2: Model 1 plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol
Model 3: Model 2 plus triglycerides, body mass index, alcohol consumption, glucose, and $\log_e$ high sensitivity C-reactive protein
Model 4: Model 3 plus $\log_e$ urinary albumin excretion and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation)

Table 4 Prospective association of NGAL with risk of cardiovascular disease outcomes using complex survey analyses

NGAL	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Cardiovascular disease									
Q1 – Q2	131 / 2639	ref		ref		ref		ref	
Q3	89 / 1319	1.27 (0.95 to 1.70)	0.107	1.19 (0.88 to 1.61)	0.249	1.14 (0.84 to 1.54)	0.414	1.06 (0.77 to 1.45)	0.724
Q4	118 / 1317	1.53 (1.17 to 2.01)	0.002	1.36 (1.04 to 1.80)	0.027	1.21 (0.91 to 1.61)	0.194	0.99 (0.73 to 1.35)	0.960
Coronary heart disease									
Q1 – Q2	99 / 2639	ref		ref		ref		ref	
Q3	66 / 1319	1.24 (0.88 to 1.73)	0.218	1.20 (0.85 to 1.69)	0.312	1.17 (0.82 to 1.66)	0.379	1.09 (0.76 to 1.57)	0.624
Q4	80 / 1317	1.28 (0.92 to 1.78)	0.136	1.14 (0.82 to 1.60)	0.437	1.07 (0.75 to 1.51)	0.721	0.86 (0.59 to 1.27)	0.459
Stroke									
Q1 – Q2	36 / 2639	ref		ref		ref		ref	
Q3	21 / 1319	1.14 (0.64 to 2.02)	0.653	1.06 (0.59 to 1.90)	0.852	1.01 (0.55 to 1.84)	0.976	0.97 (0.53 to 1.79)	0.922
Q4	34 / 1317	1.74 (1.05 to 2.86)	0.030	1.64 (0.98 to 2.73)	0.059	1.36 (0.78 to 2.37)	0.280	1.21 (0.68 to 2.15)	0.512

CI, confidence interval; HR, hazard ratio; NGAL, neutrophil gelatinase-associated lipocalin
Model 1: Age and sex
Model 2: Model 1 plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol
Model 3: Model 2 plus triglycerides, body mass index, alcohol consumption, glucose, and $\log_e$ high sensitivity C-reactive protein
Model 4: Model 3 plus $\log_e$ urinary albumin excretion and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation)

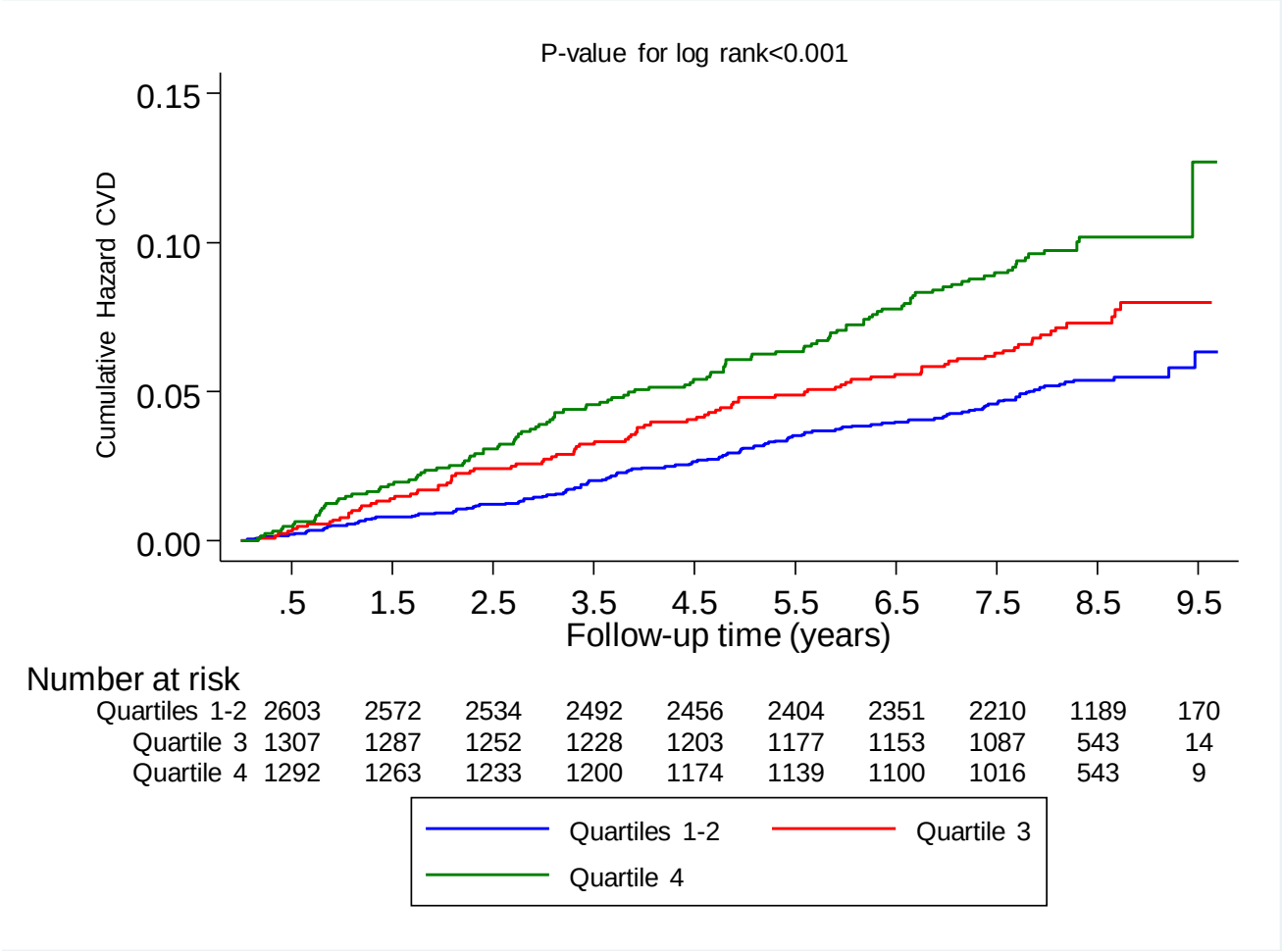
Table 5 Prospective association of NGAL with risk of cardiovascular disease outcomes (with quartile 2 of NGAL distribution as reference comparison)

NGAL	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Cardiovascular disease									
Q1	70 / 1326	1.20 (0.85 to 1.69)	0.308	1.21 (0.85 to 1.70)	0.286	1.21 (0.86 to 1.71)	0.273	1.23 (0.87 to 1.74)	0.240
Q2	61 / 1313	ref		ref		ref		ref	
Q3	89 / 1319	1.43 (1.03 to 1.98)	0.032	1.37 (0.98 to 1.89)	0.062	1.30 (0.94 to 1.81)	0.114	1.25 (0.90 to 1.74)	0.191
Q4	118 / 1317	1.62 (1.19 to 2.22)	0.002	1.48 (1.08 to 2.03)	0.014	1.32 (0.96 to 1.82)	0.088	1.16 (0.83 to 1.62)	0.396
Coronary heart disease									
Q1	47 / 1326	1.08 (0.73 to 1.60)	0.713	1.09 (0.73 to 1.62)	0.677	1.09 (0.73 to 1.62)	0.673	1.07 (0.72 to 1.59)	0.753
Q2	52 / 1313	ref		ref		ref		ref	
Q3	66 / 1319	1.32 (0.91 to 1.93)	0.145	1.30 (0.89 to 1.90)	0.181	1.27 (0.86 to 1.86)	0.224	1.20 (0.81 to 1.77)	0.358
Q4	80 / 1317	1.37 (0.95 to 1.98)	0.088	1.26 (0.87 to 1.83)	0.227	1.17 (0.80 to 1.72)	0.409	1.02 (0.68 to 1.54)	0.917
Stroke									
Q1	23 / 1326	0.56 (0.28 to 1.11)	0.097	0.56 (0.28 to 1.11)	0.095	0.54 (0.27 to 1.08)	0.080	0.54 (0.27 to 1.07)	0.076
Q2	13 / 1313	ref		ref		ref		ref	
Q3	21 / 1319	0.88 (0.49 to 1.60)	0.686	0.82 (0.45 to 1.50)	0.526	0.75 (0.41 to 1.37)	0.349	0.72 (0.39 to 1.33)	0.288
Q4	34 / 1317	1.21 (0.71 to 2.08)	0.485	1.14 (0.65 to 1.98)	0.646	0.89 (0.50 to 1.57)	0.688	0.80 (0.43 to 1.49)	0.479

CI, confidence interval; HR, hazard ratio; NGAL, neutrophil gelatinase-associated lipocalin
Model 1: Age and sex
Model 2: Model 1 plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol
Model 3: Model 2 plus triglycerides, body mass index, alcohol consumption, glucose, and $\log_e$ high sensitivity C-reactive protein
Model 4: Model 3 plus $\log_e$ urinary albumin excretion and estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation)

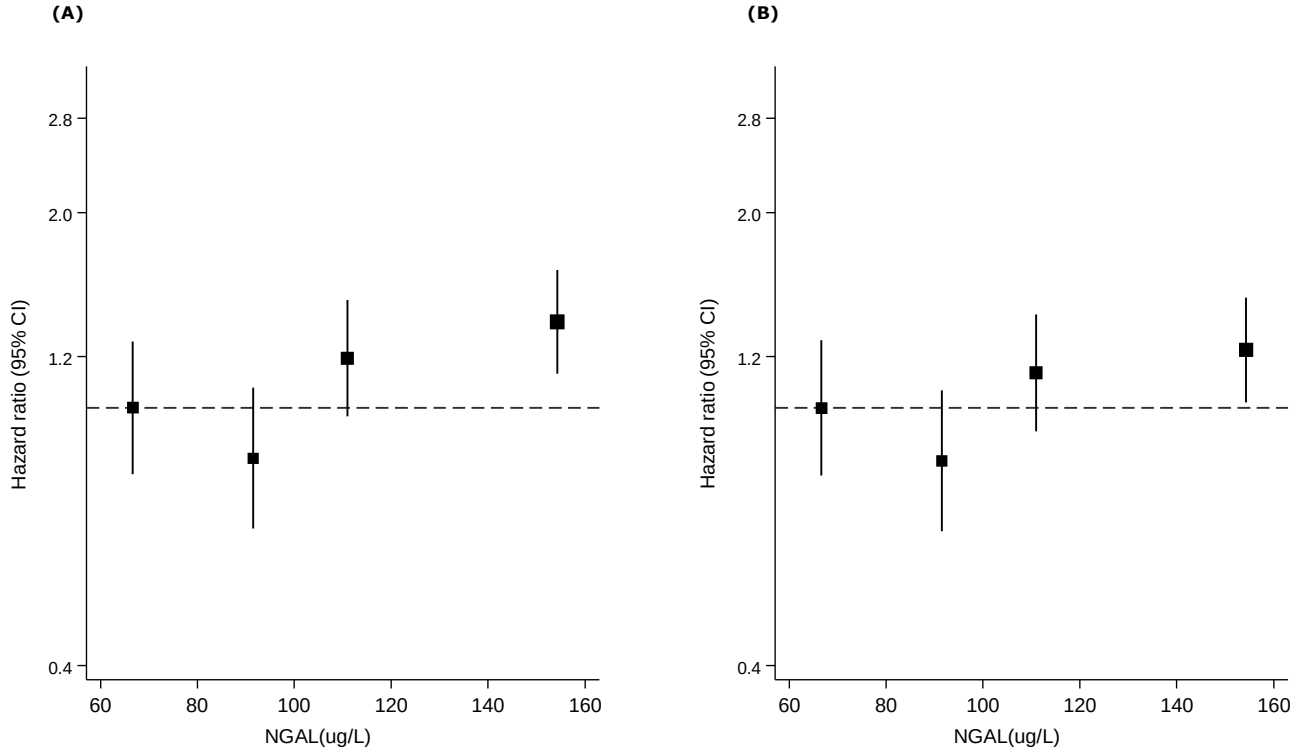
Figure legends

Figure 1 Cumulative Kaplan-Meier curves for cardiovascular disease during follow-up according to quartiles of NGAL



CVD, cardiovascular disease; NGAL, Neutrophil gelatinase-associated lipocalin

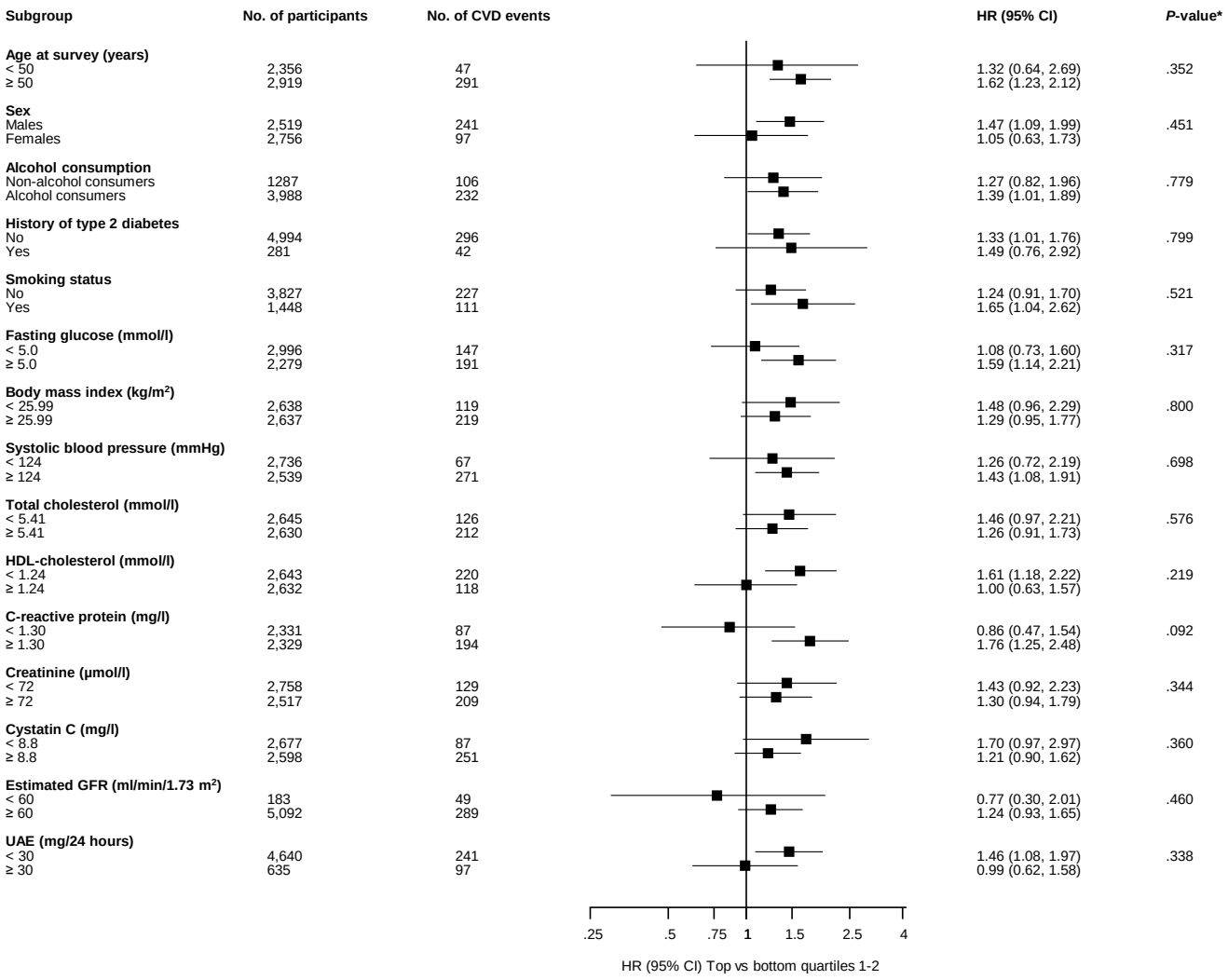
Figure 2 Hazard ratios for incident cardiovascular disease, by baseline concentrations of plasma NGAL using floating absolute risks



A, Hazard ratios were adjusted for age and sex; **B**, adjustment in A plus smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol

NGAL, Neutrophil gelatinase-associated lipocalin

Figure 3 Hazard ratios for NGAL and cardiovascular disease risk by several participant level characteristics



Hazard ratios were adjusted for age, sex, smoking status, history of type 2 diabetes, systolic blood pressure, total cholesterol, and high-density lipoprotein-cholesterol; CI, confidence interval (bars); CVD, cardiovascular disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HR, hazard ratio; hs, high sensitivity; NGAL, Neutrophil gelatinase-associated lipocalin

*, *P*-value for interaction; cut-offs used for fasting glucose, body mass index, systolic blood pressure, total cholesterol, HDL-cholesterol, hsCRP, creatinine, and cystatin C are median values.